The PerFect™ Lipid Optimizer Kit for Maximizing Lipid-Mediated Transfection of Eukaryotic Cells

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BioTechniques 22:982-987 (May 1997)

ABSTRACT

The transfection efficiencies of a panel of eight uniquely different lipid reagents has been evaluated with two other commercially available lipids for use in transfecting a diversity of eukaryotic cell lines. The PerFect[®] lipids are available individually or together in an optimization panel format that can be tested in any given cell line, enabling one to evaluate the optimal lipid for transfecting each individual cell line. Our results demonstrate that no single lipid is optimal for plasmid transfection over a broad range of cell types, thus emphasizing the need for multiple unique lipid reagents and a simple format for testing their transfection efficiency on a given cell type.

INTRODUCTION

The ability to transfect eukaryotic cells provides a very powerful tool for characterizing the function and control of genes of interest (1,6). The most frequently used methods to introduce DNA into cells are DEAE-dextran, calcium phosphate, electroporation and lipid-mediated transfection (2–5,7). In this paper, we describe the transfection efficiency in a variety of cell lines of a panel of eight different lipid reagents available from Invitrogen and two other commercially available lipid reagents. The eight PerFect™ lipid reagents are either a single cationic lipid, a combination of two cationic lipids or a combination of a cationic lipid with the neutral

lipid, L-dioleoyl phosphatidylethanolamine (DOPE) (Table 1).

The PerFect Lipid Transfection Kit provides a panel of eight different lipid reagents for introduction of nucleic acids into eukaryotic cells. It is essential to test the entire panel of eight lipids in order to find which lipid is best for each cell line. In the six cell lines on which we have focused, it is very clear that different lipids are optimal for different cell lines (Tables 2 and 3). The PerFect Lipid Transfection Kit provides reagents and protocols to test the panel of eight lipids in their cell line of choice. The kit includes pcDNA3.1™/His/LacZ as a test plasmid to screen the eight lipids. In pcDNA3.1/His/LacZ, the *E. coli* gene encoding β -galactosidase (β -gal) is expressed in mammalian cells using the immediate-early promoter from cytomegalovirus (CMV). A successful transfection will result in β -gal expression that can easily be detected by fixing and incubating the cells with 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside (X-gal) substrate.

MATERIALS AND METHODS

Materials and Cell Lines

All mammalian cell lines used in this study were obtained from ATCC (Rockville, MD, USA) except for SNB19, which was obtained from Genta (San Diego, CA, USA). All tissue culture reagents were from BioWhittaker (Walkersville, MD, USA). Reagents used to fix and stain the cells were from the

Table 1. Information on PerFect Lipids

Lipid No.	Composition
1	1:1 mixture of two cationic lipids
2	Single cationic lipid
3	1:1 mixture of a cationic lipid and DOPE
4	1:1 mixture of two cationic lipids
5	1:1 mixture of two cationic lipids
6	1:1 mixture of a cationic lipid and DOPE
7	1:1 mixture of a cationic lipid and DOPE
8	Single cationic lipid

Table 2. Cell Lines Transiently Transfected Using PerFect Lipids

Cell line	Cell Type	Origin
COS-7	Fibroblasts	Monkey
HeLa	Epithelial	Human
CHO	Fibroblasts	Hamster
SK-BR-3	Breast Carcinoma	Human
NIH3T3	Fibroblasts	Mouse
SNB19	Neuronal Glial	Human

β -gal Staining Kit (Invitrogen, San Diego, CA, USA). The medium used for transfections was OPTI-MEM® (Life Technologies, Gaithersburg, MD, USA). The lipid reagents in the PerFect Lipid Optimizer Kit were from Invitrogen. The other two commercially available lipids come from two separate companies and will be denoted A and B.

To test the ability of the eight different PerFect Lipids to transfect cells, six cell lines were utilized (Table 2 lists the cell lines, their types and their origins). Included in the six are three fibroblast lines: COS-7 (monkey), CHO (hamster) and NIH3T3 (mouse); 1 cervical epithelial line: HeLa (human); 1 breast carcinoma line: SK-BR-3 (human); and 1 neuronal line: SNB19 (human).

Maintenance of Cell Lines and Culture

COS-7, NIH3T3 and SNB19 cells were maintained in Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum (FBS). HeLa cells were maintained in Eagle's minimal essential medium (EMEM) containing 10% FBS and L-glutamine (20 mM). SK-BR-3 cells were maintained in RPMI 1640 medium containing 10% FBS. CHO cells were maintained in Ham's F-12 medium containing 10% FBS.

Transfection of Mammalian Cells with PerFect Lipid Reagents

Using the appropriate complete media for each cell line, the cells were seeded into 12-well plates in complete media and allowed to recover for 12–24 h. Transfections were performed on cells that were 50%–60% confluent. For most cell lines, seeding 5×10^4 cells/well gave approximately 50% confluency the following day. The β -gal reporter plasmid was diluted to a final concentration of 4 μ g/mL in OPTI-MEM in a polystyrene tube. Each lipid reagent was diluted to a final concentration of 24 μ g/mL in OPTI-MEM in another polystyrene tube. This gave a 6:1 ratio of lipid to plasmid by weight. The DNA and lipid mixtures were combined to make 2 mL of transfection mixture. The cells were washed with phosphate-buffered saline (PBS), and 1 mL of transfection mixture was added to each well. Each of the eight lipid reagents was tested in duplicate. The cells were returned to the incubator for 4 h, after which the transfection mixture was removed and replaced with normal culture medium (1 mL/well). The cells were returned to the incubator for 24 h before they were fixed in 2% formaldehyde, 0.2% glutaraldehyde in PBS (pH 7.3) for 10 min and stained with X-gal reagent [(1 mg/mL X-gal, 4 mM potassium ferricyanide, 4 mM potassium ferrocyanide, 2 mM magnesium chloride

Figure 1. Transient transfection of six different cell lines using the PerFect Lipids and two popular commercially available lipids. Graphs A–F represent the results of transfections of six separate cell lines (denoted above each graph) with a panel of lipid reagents. Five $\times 10^4$ cells were transfected in duplicate in each experiment with a CMV-driven reporter plasmid expressing β -gal. Twenty-four hours after transfection, cells were fixed, stained with X-gal and the transfection efficiency was determined as the percentage of cells that stained blue. Each data point reflects between 4 and 18 separate experiments. The transfection efficiency is expressed as the percent of cells staining blue after incubating with the X-gal substrate + the standard error of the mean. The numbers below the X-axis represent the lipids utilized, bars numbered 1–8 represent PerFect Lipids 1–8, while bar No. 9 represents results with commercially available Lipid A, and No. 10 denotes the commercially available Lipid B.

hexahydrate in PBS (without cations) pH 7.4)] to assay for β -gal expression as instructed in the β -gal Staining Kit. Cells that had been transfected successfully with a *LacZ* reporter plasmid and that were expressing β -gal, developed blue color. Transfection efficiency was analyzed by determining the percentage of cells that were blue. Cells were transfected with commercial lipid reagents A and B by following the protocol recommended by the manufacturers of each.

Optimization of Generic Transfection Protocol

The generic protocol of a 6:1 lipid:DNA ratio and a 4-h transfection time worked well for most cell lines tested. However, certain parameters can be optimized to obtain more efficient transfection of specific cell lines. Two parameters to optimize, if the generic protocol does not work well, are the lipid:DNA ratio and the length of transfection time.

A series of experiments were done to test these parameters. To optimize the lipid:DNA ratio, we kept the length of transfection time at 4 h and varied the ratio of lipid:DNA using ratios of 3:1, 6:1, 9:1 and 12:1. To optimize the length of transfection time, we kept the 6:1 lipid:DNA ratio constant and transfected the cells for 1, 2, 3, 4, 8 and 24 h.

RESULTS AND DISCUSSION

Composition of the PerFect Lipid Reagents

The PerFect Lipid Transfection Kit contains eight proprietary lipid reagents developed at Genta Inc. Each of the eight reagents are uniquely different. They are composed of either a single unique cationic lipid, a combination of two different cationic lipids or a combination of a single cationic lipid with the neutral lipid, DOPE. Table 1 lists the composition of each of the reagents.

Transfection Efficiency of the PerFect Lipid Reagents in Different Cell Lines

Figure 1, A–E illustrates the transfection efficiency of the eight PerFect Lipids on 5 of the 6 cell lines tested using the generic protocol, which suggests a 6:1 lipid:DNA ratio and a 4-h transfection time. One exception was the NIH3T3 cells (Figure 1F), which required three times as much DNA:lipid at a 6:1 ratio and a 24-h transfection time. The transfection efficiencies of these lipids were compared with two other commercially available lipid reagents, denoted Lipid A and Lipid B.

In Figure 1A, we demonstrate that each of the different PerFect Lipids display varying efficiencies of transfection in a given cell line. Efficiency was measured by transfecting the cells with a *LacZ* reporter plasmid. After 24 h of expression, the cells were fixed, exposed to X-gal and the cells expressing β -gal were quantitated. The numbers 1–8 in Figure 1A, and subsequent figures, correspond to PerFect Lipids 1–8. Notice that when COS-7 cells were transfected with PerFect lipids 1, 6 or 7, the percent of β -gal-expressing cells were 17.8, 16.8 and 17.3, respectively. But, when PerFect Lipids 3 and 5 were used, only 7.26% and 7.30%, respectively, of the cells were expressing β -gal. When Lipid A (No. 9) was used to transfect the COS-7 cells, only 2.44% transfection efficiency was observed. This transfection efficiency was even less than the

Table 3. Transfection Efficiency of PerFect Lipids in a Panel of Cell Lines

Cell line	Lipid No.	% Blue cells
		mean $\pm$ standard error of the mean
COS-7	1	17.83 $\pm$ 6.4 (<i>n</i> = 10) range (7.0%–28.5%)
	6	16.8 $\pm$ 8.3 (<i>n</i> = 18) range (6.1%–32.0%)
	7	17.3 $\pm$ 9.51 (<i>n</i> = 9) range (4.6%–27.0%)
HeLa	1	14.8 $\pm$ 5.4 (<i>n</i> = 18) range (7.1%–25.0%)
	2	17.1 $\pm$ 5.6 (<i>n</i> = 18) range (7.6%–32.0%)
CHO	4	34.6 $\pm$ 6.2 (<i>n</i> = 7) range (28.0%–49.0%)
	7	31.6 $\pm$ 8.3 (<i>n</i> = 9) range (13.0%–41.0%)
	8	43.0 $\pm$ 8.2 (<i>n</i> = 4) range (32.0%–55.0%)
SK-BR-3	7	25.9 $\pm$ 12.1 (<i>n</i> = 4) range (12.2%–38.0%)
NIH3T3	1	8.9 $\pm$ 2.6 (<i>n</i> = 10) range (4.8%–14.2%)
	3	8.7 $\pm$ 2.8 (<i>n</i> = 5) range (5.0%–12.0%)
SNB19	3	17.8 $\pm$ 5.3 (<i>n</i> = 8) range (6.4%–24.0%)
	7	15.6 $\pm$ 7.1 (<i>n</i> = 12) range (6.9%–30.0%)
	8	14.85 $\pm$ 6.2 (<i>n</i> = 10) range (8.0%–26.9%)

least-efficient PerFect Lipids 3 and 5. Lipid B (No. 10) worked as well as the moderately efficient PerFect Lipids 3 and 5, with the percent transfected cells being 7.33%.

A different pattern was seen in Figure 1B, when HeLa cells were transfected with the eight PerFect Lipids (1–8) and Lipids A and B (Nos. 9 and 10, respectively). In this cell line, PerFect Lipid 2 gave the highest efficiency with 17.1% blue cells. In contrast, PerFect Lipid 5 had a transfection efficiency of only 5.8%. While Lipid A was actually comparable to our best lipid in transfection efficiency, Lipid B yielded the lowest transfection efficiency with 4.9% blue cells.

In CHO cells (Figure 1C), PerFect Lipid 8 gave the highest

Figure 2. Comparison of the efficiency of lipid-mediated transfection of CHO cells with multiple uniquely different lipid reagents in suspension vs. attached to culture dishes. CHO cells were transfected with the 10 different lipids in suspension or attached cultures, and the transfection efficiency was determined by counting the percentages of cells expressing the reporter gene β -gal. Paired bars labeled 1–8 represent values obtained with PerFect Lipids 1–8, respectively, and 9 and 10 represent commercially available Lipids A and B, respectively.

transfection efficiency with 43% blue cells. Note that when PerFect Lipid 8 was used in COS-7 cells (Figure 1A), only 9.6% cells were transfected, and in the HeLa cells (Figure 1B), there were only 7.0% blue cells. PerFect Lipid 8 is clearly the optimal lipid for transfection of CHO cells, though PerFect Lipids 2 (21.9%), 4 (34.6%) and 7 (31.6%) also exhibited transfection efficiencies that were more than adequate in this cell line. Conversely, when PerFect Lipids 5 and 6 were used to transfect CHO cells, the transfection efficiencies were only 3.5% and 5.6%, respectively. Lipid B had a transfection efficiency that was moderate, while Lipid A had an efficiency of only 2.5%, the lowest of all the lipids tested. This low efficiency, when compared to the high efficiency Lipid A had in the HeLa cells (Figure 1B), demonstrates that Lipid A works more optimally in some cell types than others, as do each of the 8 PerFect Lipids.

Figure 1D illustrates the results of experiments in which SK-BR-3 cells were transfected with the same lipid panel. Notice PerFect Lipid 7 gave a transfection efficiency of 25.9%. It is clearly the optimal lipid to use when transfecting this cell line. The transfection efficiencies using PerFect Lipids 1 (10.8%), 2 (6.9%), 3 (9.8%), 4 (11.3%), 8 (10%) and Lipid A (13.2%) were moderate. Lipid B (2.3%) and PerFect Lipid 5 (1.3%) yielded the lowest transfection efficiencies.

In Figure 1E, SNB19 cells were tested using the PerFect Lipids with the generic protocol. For this cell line, PerFect Lipids 3 (17.8%), 7 (15.6%) and 8 (14.9%) had the highest transfection efficiencies, though PerFect Lipids 1 (10.4%) and 2 (10.0%) also had efficiencies that were quite reasonable. Lipid B had a moderate transfection efficiency in SNB19 cells (9.4%), while Lipid A had the lowest percent blue cells (4.0%).

When NIH3T3 cells were transfected with the PerFect lipids, some parameters had to be optimized. Efficient transfection of this cell line did not occur when the generic protocol of a 6:1 ratio of lipid:DNA and a 4-h transfection time was used. None of the cells were expressing β -gal. Parameters optimized included the amount of DNA and lipid used and the length of transfection time. The ratio of lipid:DNA was kept at 6:1, but 3 times as much DNA and lipid were used, and the length of transfection time was extended from 4 to 24 h. Figure 1F presents the results of the NIH3T3 transfection using PerFect Lipids with the optimized protocol. Lipids A and B were used according to the protocol recommended by the manufacturer of each, but as with the PerFect Lipids, 3 times as much DNA and lipid were used. Under these optimized conditions, PerFect Lipids 1 (8.9%) and 3 (8.7%) gave the highest transfection efficiencies. PerFect Lipids 4 and 8 were tested, but the transfection efficiencies obtained using these lipid reagents were actually nonexistent. The other PerFect Lipids all had transfection efficiencies that fell somewhere within a range of 2.0% to 6.0% blue cells, as did Lipid A (2.0% blue cells) and Lipid B (4.1% blue cells).

Table 3 illustrates that different PerFect Lipids are optimal in different cell lines. This is due to the unique composition of each of these lipids. The cell lines listed in Table 3 were all transfected with each of the PerFect Lipids. Table 3 lists only the lipids that gave optimal results for each cell line. When PerFect Lipid 8 was used to transfect CHO cells, the mean percent blue cells was 43.0%, while when the same lipid was used in SNB19 cells, the mean percent blue cells was 14.85%. Though both of these transfection efficiencies are good, it is clear that CHO cells are more receptive to

Table 4. Optimization of the Generic Protocol

A. Optimizing Lipid:DNA ratio					
CHO (Lipid No. 7 ^a)			HeLa (Lipid No. 2 ^a)		
Lipid:DNA Ratio	% Blue Cells		Lipid:DNA Ratio	% Blue Cells	
3:1	2.4	1.3	3:1	<1	1.7
6:1	39	43	6:1	19.4	27.8
9:1	15.9	29	9:1	15.2	18.6
12:1	Cells very sparse		12:1	Cells very sparse	
^a Length of transfection time was 4 h.					
B. Optimizing Length of Transfection Time					
CHO (Lipid No. 4 ^b)			HeLa (Lipid No. 1 ^b)		
Time (hours)	% Blue Cells		Time (hours)	% Blue Cells	
1	1.3	1.2	1	2.9	1.6
2	2.7	12.5	2	5.8	7.4
3	42.5	29.5	3	10.1	16.0
4	38.6	44.6	4	18.5	18.0
8	Cell very sparse		8	Cells very sparse	
^b Lipid:DNA ratio was 6:1.					

transfection with lipids than SNB19 cells. PerFect Lipid 7 worked quite well in COS-7 (17.3%), SNB19 (15.6%), SK-BR-3 (25.9%) and CHO (31.6%). Clearly, this lipid can be used to optimally transfect multiple cell lines. Of note, PerFect Lipids 1 (14.8%) and 2 (17.1%) gave the best transfection efficiencies in HeLa cells, while in NIH3T3 cells, PerFect Lipids 1 (8.9%) and 3 (8.7%) gave optimal results. These data suggest that although some of the lipids can be utilized to transfect multiple cell types, these lipids may be completely ineffective in other cell lines. Clearly, the availability of a panel of uniquely different lipids for analyzing the optimal lipid for transfecting a given cell line will greatly facilitate experiments involving gene transfer.

In COS-7 cells, the three PerFect Lipids that performed optimally (Nos. 1, 6 and 7) all had transfection efficiencies that ranged from 16.8%—17.8%. In the CHO cells, the three optimal lipids (Nos. 4, 7 and 8) had efficiencies that ranged from 31.6%—43.0%. In NIH3T3 cells, the transfection efficiencies of the two best PerFect Lipids (Nos. 1 and 3) were 8.9% and 8.7%, respectively. Additionally, in the HeLa cells, the transfection efficiencies for PerFect Lipids 1 (14.8%) and 2 (17.1%) were also very similar. These data suggest that transfection efficiency is not only influenced by the type of lipid reagent used, but also by the individual cell line being transfected. As the data in Table 3 clearly demonstrate, transfection efficiencies will normally vary from transfection to transfection. For example, notice that when PerFect Lipid 7 was used to transfect COS-7 cells, the mean percent blue cells that were counted, from a total of nine separate transfections was $17.3\% \pm 9.51\%$, while the range of percent blue cells, from the same nine transfection reactions, was from 4.6% to 27.0%. Figure 2 depicts a graphic example of the differences in transfection efficiency of each of the lipids utilized in this study. Note that this single cell line is differentially transfected with each of the lipids utilized.

Optimization of the Generic Protocol

The generic transfection protocol for the PerFect Lipids

suggests a 6:1 lipid:DNA ratio and a 4-h transfection time. To demonstrate the efficiency of this protocol, the ratio of DNA to lipid and the length of transfection time were each varied. CHO and HeLa cells were used for the optimization studies. Table 4A lists what was observed when the lipid:DNA ratio was varied and the length of transfection time was held constant at 4 h. For both the CHO and HeLa cells, the generic protocol of 6:1 lipid:DNA ratio was optimal. When PerFect Lipid 7 was used to transfect CHO cells in duplicate at a ratio of 3:1, the percent blue cells were only 2.4% and 1.3%. But, when a ratio of 6:1 was used, the percentages of blue cells observed were 39% and 43%. When the lipid:DNA ratio was increased to 9:1, the percent blue cells dropped to 15.9% and 29%, while at a 12:1 lipid:DNA ratio, the cells were actually too sparse on the plate to count. This same pattern was seen when PerFect Lipid 2 was used to transfect HeLa cells at variable lipid:DNA ratios. The toxicity of this higher concentration of lipid was the reason for the decrease in transfection efficiency.

Table 4B presents the results obtained when the length of transfection time was varied from 1, 2, 3, 4 and 8 h, while the lipid:DNA ratio of 6:1 was held constant. CHO and HeLa cells were each transfected with one of their optimal lipids, PerFect Lipid 4 for CHO and PerFect Lipid 1 for HeLa. It is very clear that a 3- or 4-h transfection time is optimal for both CHO and HeLa cells. Time points under 3 h produced a considerable decrease in percent blue cells. This is most likely due to the fact that the length of transfection time was not long enough to allow the lipid-DNA complex to penetrate the cell wall and deliver the DNA into the cell. At 8 h, the cells became too sparse to count, which again illustrates the toxic effect that the lipids have over time on some cells.

Some cell lines are not receptive to transfection by lipids under any conditions. Jurkat cells, a suspension cell line derived from human acute T-cell leukemia, were transfected with pcDNA3.1/His/LacZ, using the PerFect Lipids with the generic protocol. None of the lipids performed well; although, lipids 5 and 7 demonstrated the best transfection efficiency with a meager 1%–5% blue cells.

To examine if this phenomenon was suspension-cell-relat-

ed, we cultured CHO suspension cells by growing them in CHO SFM II media (Life Technologies). Since adherent CHO cells had the highest transfection efficiencies of the six cell lines tested (Figure 1, A–F), they would be a reliable line to test in suspension. The cells were transfected with pcDNA3.1/His/LacZ using the generic protocol of a 6:1 lipid:DNA ratio and a 4-h transfection time. After 24 h of expression, they were assayed for β -gal activity (Figure 2). The CHO suspension cells transfected remarkably well with PerFect Lipids 1 (33%), 4 (36%) and 6 (34%). Note that in the adherent CHO cells, lipids 4, 7 and 8 gave optimal transfection efficiencies (Table 3). Though lipid 4 worked quite well in both the adherent and suspension CHO cells, lipids 1 and 6, which performed best in suspension cells, gave some of the lowest efficiencies in the adherent cells. These data demonstrate that cell morphology, as well as cell type, can influence a cell's ability to be transfected using lipids. It also affirms the necessity of utilizing a panel of eight uniquely different lipids to analyze the optimal lipid for transfecting a given cell line.

Collectively, these results suggest that, aside from NIH3T3 cells, which are very difficult to transfect, and required an increased concentration of lipid:DNA and 24-h transfection time, the generic protocol of a 6:1 lipid:DNA ratio and a 4-h transfection time is optimal for several cell lines.

SUMMARY

We have demonstrated the utility of the PerFect Lipid Transfection Kit for optimizing lipid-mediated delivery of plasmids into different cell lines of interest. By utilizing the panel of eight uniquely different lipids, transfection parameters and lipid choice can be optimized to obtain the best plasmid introduction possible, in any given cell line. Each of the eight lipids have varying efficiencies in any given cell line, with different lipids being optimal for different cell lines. Because of this characteristic, the PerFect Lipid Panel will, in general, contain a specific lipid that will be efficient for transfecting most cell lines. To our knowledge, no single lipid (in the PerFect Panel or available elsewhere) is optimal for plasmid transfection over a very broad range of cell types, emphasizing the need for multiple unique lipid reagents and a simple format for testing their transfection efficiency on a given cell type.

ACKNOWLEDGMENTS

We would like to thank the other members of the Research and Development laboratory and others elsewhere at Invitrogen for helpful comments and suggestions on the work and the manuscript. We would like to thank Doreen Crawford for her excellent assistance with the figures. PerFect Lipids were synthesized and developed at Genta Inc. by Bob D. Brown, William Daily, Brian P. Dwyer, Alexander Lebedev and David A. Schwartz. Information concerning the licensing of these lipid reagents for non-research reagent purposes can be attained by contacting Dr. Bob D. Brown at Genta Inc. (bob_brown@gsdl.compuserve.com)

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